

9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A

11. **MATERIALS AND METHODS:**

A. **Test Species:** The alga used in the test, Anabaena flos-aquae, from the Culture Centre of Algae and Protozoa, Freshwater Association, the Ferry House, Ambleside, Cumbria, UK. Stock were maintained in synthetic nutrient medium (Miller et al., 2870 lux illumination, and a temperature of $24 \pm 1^\circ\text{C}$, with orbital 100 rpm. Cool white fluorescent tubes and a continuous photoperiod used. Cultures that were growing logarithmically were used for the test.

B. **Test System:** Test vessels used were glass 250 ml conical flasks with foam stoppers. The test medium was the same as that used for culturing, with a pH between 7.2 and 7.3.

The test vessels were kept in an incubator with environmental conditions like those employed in culturing.

C. **Dosage:** Results from the preliminary range-finding test demonstrate that rates between 0.1 and 100 mg ai/l would be appropriate for a definitive test. Nominal rates of 0.1, 0.32, 1.0, 3.2, 10, 30, 100, medium control, and a blank (no algal inoculum) were used for the definitive test.

D. **Test Design:** The two highest nominal rates (100 and 32 mg ai/l) were prepared by direct addition of the test material to sterile aliquots of the stock. Lower nominal concentrations were prepared by diluting the stock. All solutions were clear. One-hundred milliliters of the test solution were placed in replicate 250 ml flasks (3 per treatment level). The control was replicated six times.

An inoculum volume of 1.490 ml was used to provide 2.0×10^4 cells/ml per flask. Cell counts were performed every 5 days using a spectro-photometer. The instrument was zeroed on the blank flask solution. After counting, the flasks were returned to their rows within the incubator.

At the start of the test, samples were taken of each test solution, the excess remaining after filling the test vessels, and were analyzed for concentration of test substance by gas chromatography (GC). At 24 hours, each blank solution was sampled and analyzed in the

manner.

The pH of the test solutions were measured at test initiation. Light intensity was measured once during the experiment and temperature was monitored continuously.

E. Statistics: For each nominal concentration, the mean of the measured concentrations from the blanks on day 0 and day 4 was calculated. The mean measured concentrations were then used as the basis for analysis. The area under the growth curve and growth rate were examined as a function of time. Probit and Dunnett's analysis were conducted on both of these parameters at day 4 and day 5.

12. **REPORTED RESULTS:** Algal cell absorbances for the control and the exposure concentrations throughout the test are given in Table 2 (attached).

Measured concentrations on day 0 were 94% to 105% of nominal while measured concentrations were between 94% and 100%. The means of measured concentrations were 0.095, 0.32, 0.95, 3.3, 9.7, 31, and 97 mg ai/l.

Increasing concentrations of molinate had increasingly inhibitory effects on growth and reproduction of Anabaena flos-aquae.

By day 5, the effect of the test material on the area under the growth curve relative to the control, ranged between 0% and 86% inhibition (Table 3, attached). The EC₅₀ was 21 mg ai/l with confidence limits of 2.1 and 210 mg ai/l.

By day 5, the effect of the test material on the growth rate, ranged between 0% and 34% inhibition (Table 4, attached). The EC₅₀ was determined to be greater than 97 mg ai/l. No confidence limits were determined.

Results from Dunnett's analysis indicated that the areas under the growth curve on day 5 at the five highest concentrations were significantly different from the controls. The lowest rate of 0.095 mg ai/l was also significantly different from the control, but since the amount of inhibition was 14% and the next highest concentration was not significantly different, the NOEC was determined to be 0.32 mg ai/l. Results from the growth rate data indicated that by day 5, the rate was not significantly different from the control. The NOEC was determined to be 97 mg ai/l.

The pH in the control and the exposure concentrations were 7.9 to 8.9, respectively, by test termination. The temperature ranged from 24.8°C to 28.8°C.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**

No conclusions were made by the authors.

Good laboratory practice and Quality Assurance Unit statements were the report indicating compliance with EPA Good Laboratory Practice under the Federal Insecticide, Fungicide, and Rodenticide Act.

14. **REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:**

- A. **Test Procedure:** The test procedure and the report were general accordance with the SEP and Subdivision J guidelines, except following deviations:

The dissolved oxygen and conductivity of the test solution measured.

The age of the organisms used for inoculum was not stated.

The light intensity was 2.87 klux. The recommended intensity

The test rates were three-fold progressions. Two-fold dilutions recommended.

The initial cell density was greater than the recommended density (20,000 vs. 3000).

Although the raw data were submitted for cell density data, the raw data for the area under the growth curve and growth rate were

No subtoxic (EC₂₅) values were reported.

- B. **Statistical Analysis:** The reviewer used a computer program to statistical analysis (attached) of the 5 day absorbance data. The NOEC, the area under the growth curve was used to determine the EC₅₀ value. Moving average and Dunnett's tests were used to determine EC and NOEC values, respectively. The results from Dunnett's were in agreement with the authors'. The reviewer obtained an EC₅₀ value that was the same as the authors', however, the confidence intervals were smaller.
- C. **Discussion/Results:** This study is scientifically sound but does not meet the guideline requirements for a Tier 2 non-target aquatic bioassay. Growth of Anabaena flos-aquae was increasingly inhibited by increasing amounts of molinate.
- D. **Adequacy of the Study:**

(1) **Classification:** Supplemental.

(2) **Rationale:** Initial cell density and test concentrations the guideline requirements.

(3) **Repairability:** No.

15. **COMPLETION OF ONE-LINER:** Yes, 6/20/91.

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